



1/28

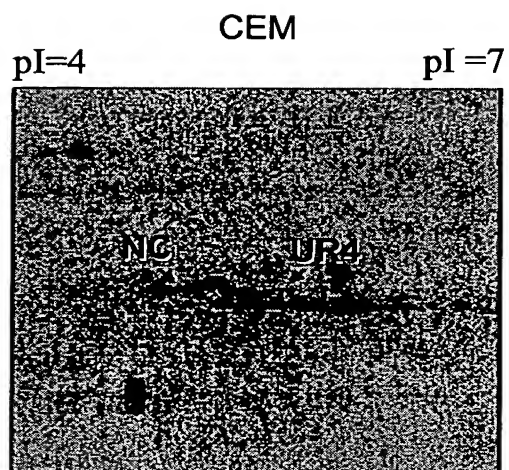


FIG. 1A

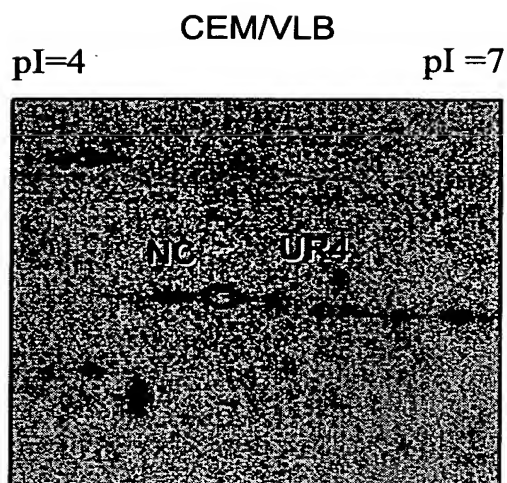


FIG. 1B

Isoform 2 Results

| ProFound -Search Result Summary | | | | Version 4.10.8 | | |
|--|-------------|---------|---|----------------|-----|---------|
| © 1997-2000 ProteoMetrics | | | | | | |
| Protein Candidates for search 20010608200436-0394208172123151 [73182 sequences searched] | | | | | | |
| Rank | Probability | Est'd Z | Protein Information and Sequence Analyse Tools (T) | % | pI | kDa ® |
| +1 | 1.0e+000 | 2.36 | gi 5729877 ref NP_006588.1 Heat shock 70 kD protein 8, heat shock 70kD protein 8 (HSP73), heat shock cognate protein, 71 kDa,heat shock 70kd protein 10(HSC71) [Homo sapiens] | 25 | 5.4 | 71.11 ® |

FIG. 2A

Search Parameters

| | |
|---|--------------------|
| Details for rank 1 candidate in search 20010608200436-0394208172123151 | |
| gi 5729877 ref NP_006588.1 heat shock 70 kD protein 8, heat shock 70kD protein 8 (HSP73), heat shock cognate protein, 71 kDa,heat shock 70kd protein 10(HSC71) [Homo sapiens] | |
| gi 13639862 ref XP_006086.2 heat shock 70kD protein 8 [Homo sapiens] | |
| gi 123648 sp P11142 HS7C HUMAN HEAT SHOCK COGNATE 71 KDA PROTEIN | |
| gi 87625 pir LA27077 dnaK-type molecular chaperone-human | |
| gi 32467 emb CAA68445.1 (Y00371)71 Kd heat shock cognate protein [Homo sapiens] | |
| gi 13273304 gb AAK17898.1 AF352832_1(AF352832) constitutive heat shock protein 70 [Homo sapiens] | |
| Sample ID | NO CHANGE [Pass 0] |
| Measured peptides | 20 |
| Matched peptides | 12 |
| Min. sequence coverage | 25% |

FIG. 2B

| <u>12 Peptides Sequenced*</u> | | Measured Avg/ Computed Error Residues Missed | | | | | |
|-------------------------------|--|--|------|----------|-------|----------|------------------------|
| | | Mass (M) | Mono | Mass | (ppm) | Start To | Cut Peptide sequence |
| -1 | | 1250.623 | M | 1250.611 | 10 | 237 246 | 0 MVNHFIAEFK |
| -2 | | 1252.592 | M | 1252.608 | -13 | 302 311 | 0 FEELNADLFR |
| -3 | | 1406.696 | M | 1406.712 | -12 | 237 247 | 1 MVNHFIAEFKR |
| -4 | | 1479.743 | M | 1479.746 | -2 | 300 311 | 1 ARFEELNADLFR |
| -5 | | 1486.756 | M | 1486.693 | 42 | 37 49 | 0 TTPSYVAFTDTER |
| -6 | | 1690.705 | M | 1690.718 | -7 | 221 236 | 0 STAGDTHLGGEFDNR |
| -7 | | 1786.968 | M | 1786.982 | -8 | 172 188 | 1 IINEPTAAAIAYGLDKK |
| -8 | | 1820.874 | M | 1820.883 | -5 | 57 72 | 1 NQVAMNPTNTVFDAGR |
| -9 | | 1837.001 | M | 1837.005 | -2 | 326 342 | 1 LDKSQIHDIIVLVGGSTR |
| -10 | | 1951.065 | M | 1951.052 | 7 | 452 469 | 1 DNNLLGKFELTGIPAPR |
| -11 | | 1980.996 | M | 1980.990 | 3 | 138 155 | 0 TVTNAVVTVPAYFNDSQR |
| -12 | | 2773.258 | M | 2773.318 | -21 | 424 447 | 0 QTQFTTYSNQPGLIQVYGER |

*25% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed.

FIG. 2C

1 MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQQNRTPSYVAFTDTERLIGDAAKNQVA
 61 **MNPTNTVFD**AKRLIGRRFDDAVVQSDMKHWPFMVNDAGRPKVQVEYKGETKSFYPEEVS
 121 SMVLTKMKEIAEAYLGKTVTNAVVTVPAYFNDSQRQATKAGTIAGLNVLRINEPTAAA
 181 IAYGLDKKVGAEARNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRMVNH
 241 **FIAEFKRKHKKDISE**NKRAVRRLRTACERAKRTLSSSTQASIEIDSLYEGIDFYTSITRA
 301 **RFEELNADLFRGT**LDPVEKALRDAKLDKSQIHDIVLVGGSTRIPKIQKLLQDFFNGKELN
 361 KSNPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLKRNTTI
 421 PTKQTQTFTTYSNDNQPGVLIQVYEGERAMTKDNNLLGKFELTGIPPAPRGVPQIEVTFDI
 481 DANGILNVSAVDKSTGKENKITITNDKGRLSKEDIERMVQEAKEYKAEDEKQRDKVSSKN
 541 SLESYAFNMKATVEDEKLQKINDEDKQILDKCNEIINWLDKNQTAKEEEFEHQQKELE
 601 KVCNPIITKLYQSAGGMPGGMPGGFPGGGAPPSSGGASSGPTIEEVD

BOLD = Peptides covered by MS MALDI analysis

FIG. 3

5/28

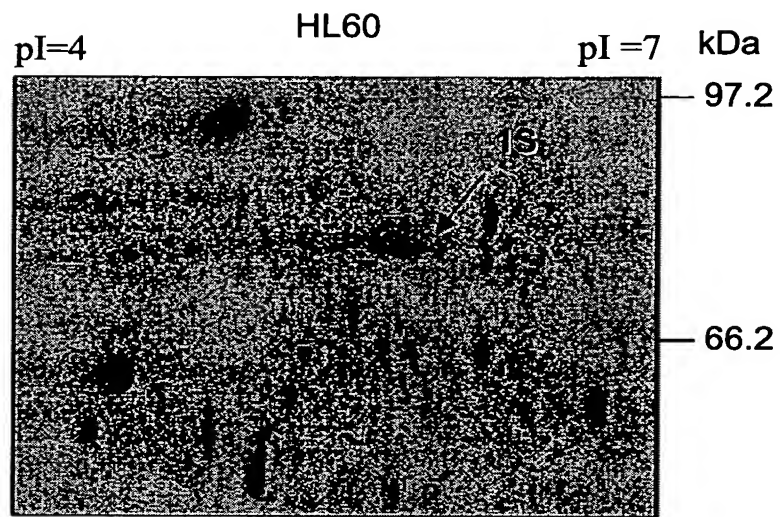


FIG. 4A

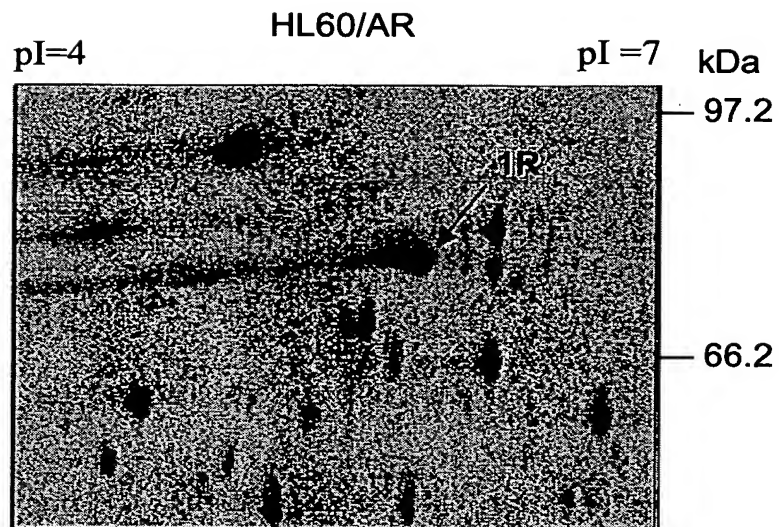


FIG. 4B

| ProFound -Search Result Summary | | | | Version 4.10.5 | | | |
|---|-------------|---------|---|------------------------------------|-----|-------|---|
| Protein Candidates for search BD36B5S9-CG5C-289FB336 [88967 sequences searched] | | | | The Rockefeller University Edition | | | |
| Rank | Probability | Est'd Z | Protein Information and Sequence Analyse Tools (T) | % | pI | kDa | ® |
| +1 | 1.0e+000 | 2.43 | T gi 5729877 ref NP_006588.1 (NM 006597) heat shock 70kd protein 8, heat shock 70kD protein 8(HSP73), heat shock cognate protein 71-kDa, heat shock 70kd protein 10 (HSC71) [Homo sapiens] | 26 | 5.4 | 71.11 | ® |

FIG. 5A

Search Parameters

| Input Summary | |
|---|---|
| Date & Time | Mon Dec 17 01 01:33 2001 UTC (Search Time: 2 80 sec) |
| Sample ID | |
| Database | NCBI nr (2001/12/11) |
| Taxonomy Category | Homo sapiens (human) |
| Protein Mass Range | 60 - 90 kDa |
| Protein pI range | 4.0 - 5.5 |
| Search for | Single protein only |
| Digest Chemistry | Trypsin |
| Max Missed Cut | 2 |
| Modifications | +C2H3ON@C(Complete), +O@M(Partial), C22H37N4O4S@K(Partial); |
| Charge State | MH+ |
| Peptide Masses (Da,Average) | |
| Tolerance(AVG) | 100.00 ppm |
| Peptide Masses (Da,Monoisotopic) | 1199.667 1253.610 1401.805 1407.785 1463.128 1480.751 1487.698 1691.729 1830.802 1838.043 1966.104 1982.001 1994.000 2231.141 2275.180 2278.081 2757.251 2774.246 2998.417 3548.224 |
| Tolerance(MON) | 100.00 ppm |
| Number of Peptides | 20 |

FIG. 5B

12 Peptides
Sequenced*

| Measured peptides | | : 20 | | | | | | | |
|---------------------------------|----------|-------|----------|-----|-----|-----|---|-------------|-------------------|
| Matched peptides | | : 12 | | | | | | | |
| Min. sequence coverage | | : 26% | | | | | | | |
| Boxed peptides are biotinylated | | | | | | | | | |
| -1 | 1198.659 | M | 1198.666 | -6 | 160 | 171 | 0 | DAGTIAGLNVL | R |
| -2 | 1252.602 | M | 1252.608 | -5 | 302 | 311 | 0 | FEELNADL | FR |
| -3 | 1406.777 | M | 1406.712 | 46 | 237 | 247 | 1 | MVNRFIAE | FKR |
| -4 | 1479.744 | M | 1479.746 | -2 | 300 | 311 | 1 | ARFEEINAD | LFR |
| -5 | 1486.690 | M | 1486.693 | -2 | 37 | 49 | 0 | TTPSYVAFT | DTER |
| -6 | 1690.721 | M | 1690.726 | 2 | 221 | 236 | 0 | STAGDTHL | GGEDFDNR |
| -7 | 1837.035 | M | 1837.005 | 16 | 326 | 342 | 1 | LDKSQTHD | IVLVGGSTR |
| -8 | 1965.096 | M | 1965.083 | 7 | 312 | 325 | 2 | GTLDPVEK | ALRDAK |
| -9 | 1980.994 | M | 1980.990 | 2 | 138 | 155 | 0 | TVTNAVVT | PAYFNDSOR |
| -10 | 1980.994 | M | 1980.956 | 19 | 518 | 533 | 3 | MVQFAEKY | KADEKQR |
| -11 | 2274.272 | M | 2274.136 | 16 | 57 | 72 | 1 | NQVAAMNP | TNTVFDAGR |
| -12 | 2773.238 | M | 2773.318 | -29 | 424 | 447 | 0 | QTQTFTT | YSDNQPGVLIQVYEGER |
| -13 | 2997.409 | M | 2997.452 | -14 | 540 | 557 | 1 | NSLESYAF | NMKATVEDEK |

Boxed peptides are biotinylated

*26% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed. Note that 2 and 4 represent the same peptide

FIG. 5C

1 MSKGPAVGIDLGTTSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAK**NQVA**
 61 **MNP****TNTV****FD****AK****RL**IGRRFDDAVVQSDMKHWPFMVNDAGRPKVQVEYKGETKSFYPEEVS
 121 SMVLTkMKEIAEAYLGKTVTNAVVTVPAYFNDSQRQATKdagTiaGLNVLRiINEPTAA
 181 IAYGLDKKVGaERNVlFDLGGGTFDVSiLTIEDGIFeVKSTAGDTHLGGEDFDNRMVNH
 241 FIAEFKRKHKKDiSENKRAVRRLRTACERAKRTLSSSTQASIEIDSLYEGIDFYTSITRA
 301 RFEELNADLFR**GTLDP****VEKAL****RD****AK**L**DK****SQ****IHD****IV**L**V**GGSTRIPKIQKLLQDFFNGKELN
 361 KSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLKRNTTI
 421 PTKQTQTFTTYSdNQPGVLIQVYEGERAMTKDNNLLGKFELTGIPPAPRGVPQIEVTFDI
 481 DANGILNVSAVDKSTGKENKITITNDKGRLSKEDIERM**VQ****E****A****E****K****Y****K****A****E****D****E****K****Q****R****D****K****V****S****K****N**
 541 **S****L****E****S****Y****A****F****N****M****K****A****T****V****E****D****E****K****L****Q****G****K****I****N****D****E****D****K****Q****K****I****L****D****K****C****N****E****I****N****W****L****D****K****N****Q****T****A****E****K****E****E****F****H****Q****Q****K****E****L**
 601 KVCNPIITKLYQSAGGMPPGGMPGGFPGGGAPPSSGGSSGPTIEEVD

BOLD = Nonbiotinylated peptides specific for HSC70
Underlined & Italics = Biotinylated peptides

FIG. 6

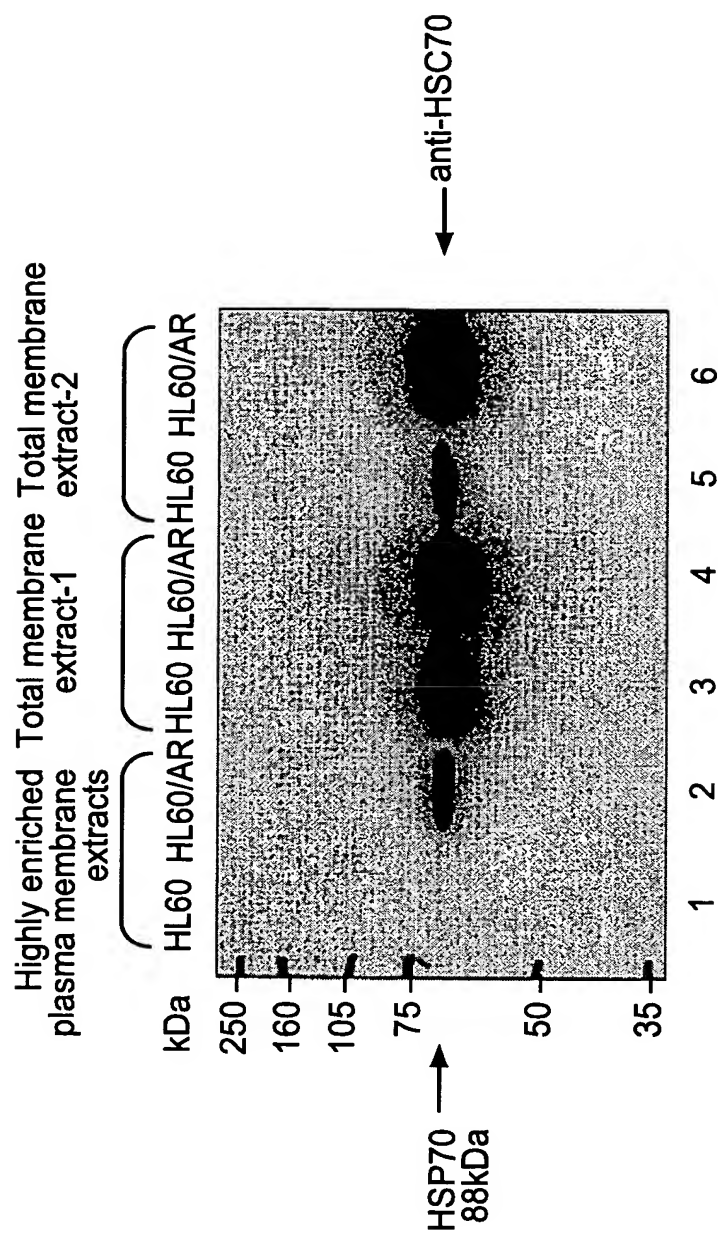


FIG. 7

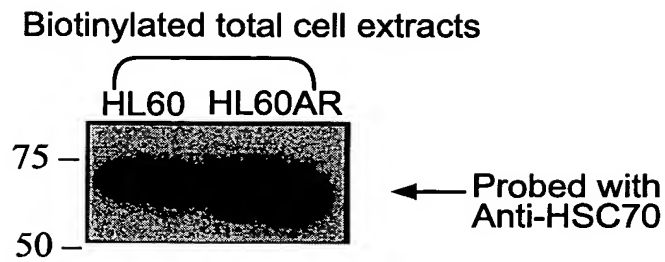


FIG. 8A

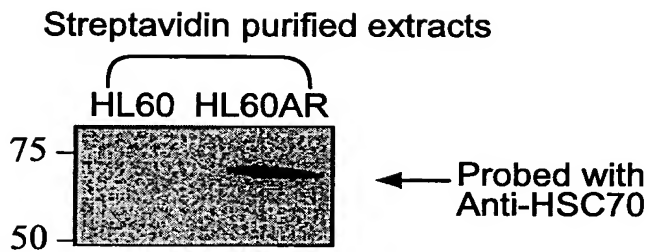


FIG. 8B

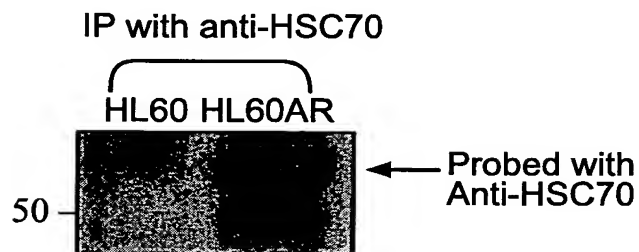


FIG. 8C

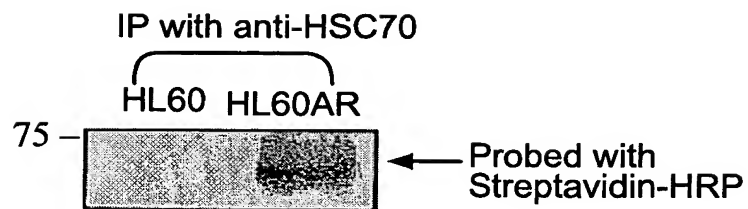


FIG. 8D

12/28

Biotinylated total cell extracts

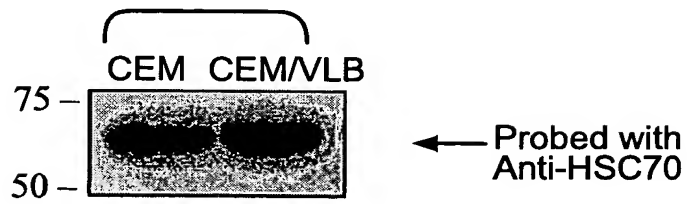


FIG. 9A

Streptavidin purified extracts

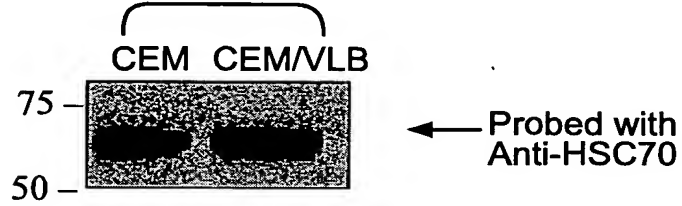


FIG. 9B

IP with anti-HSC70

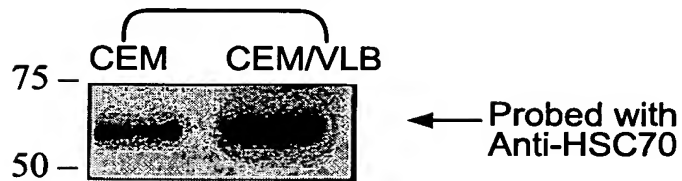


FIG. 9C

IP with anti-HSC70

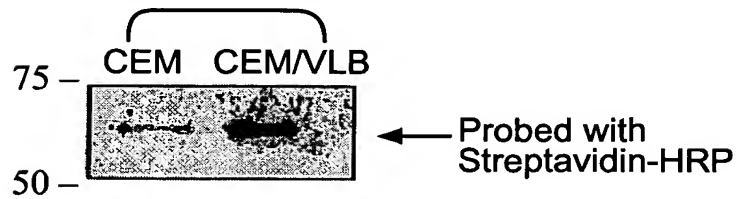


FIG. 9D

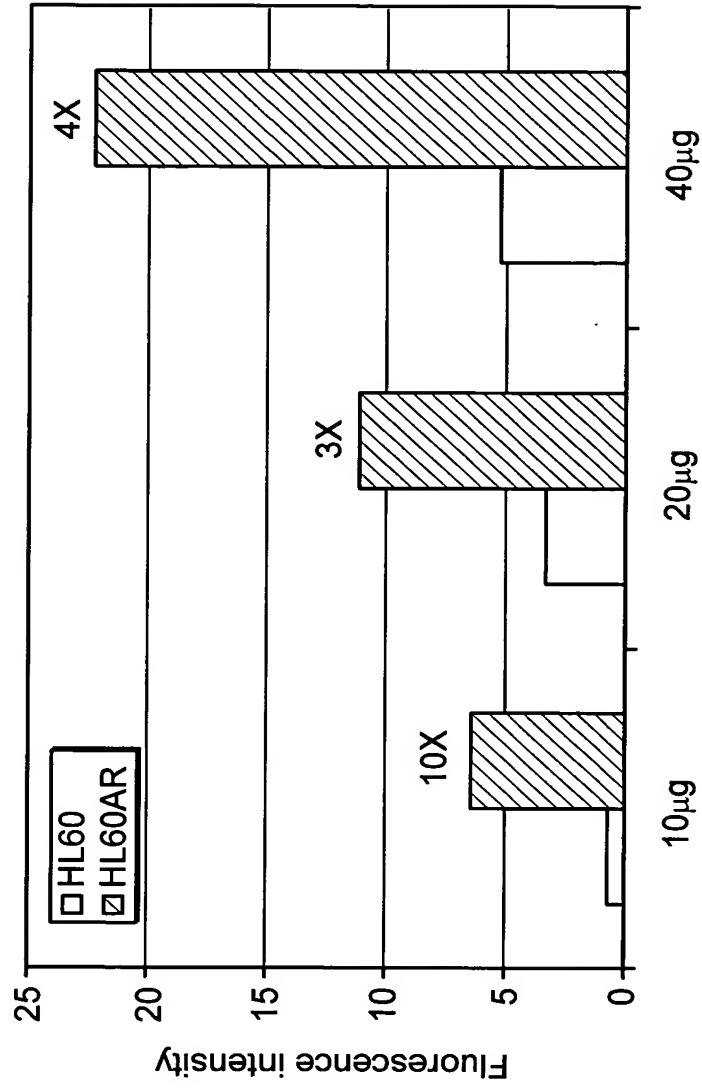


FIG. 10A

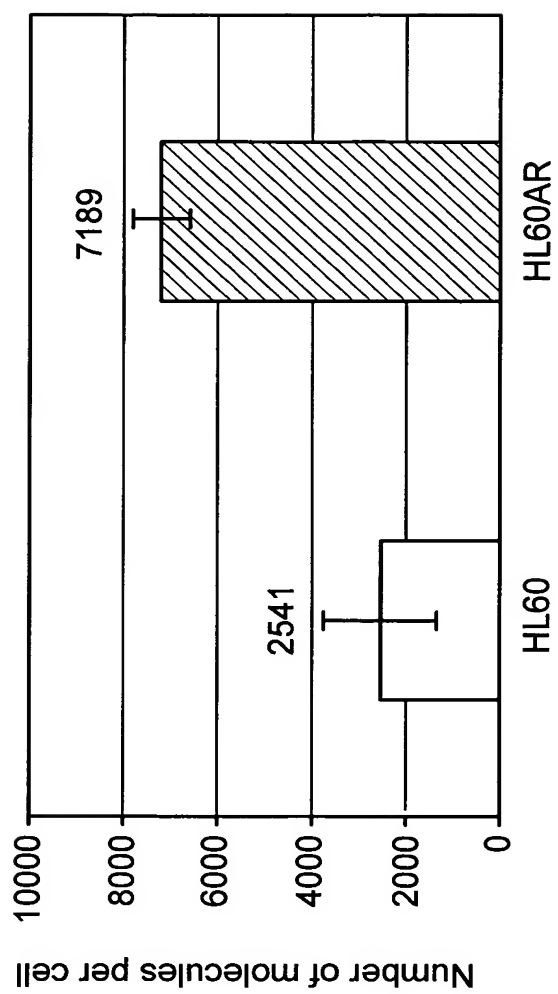


FIG. 10B

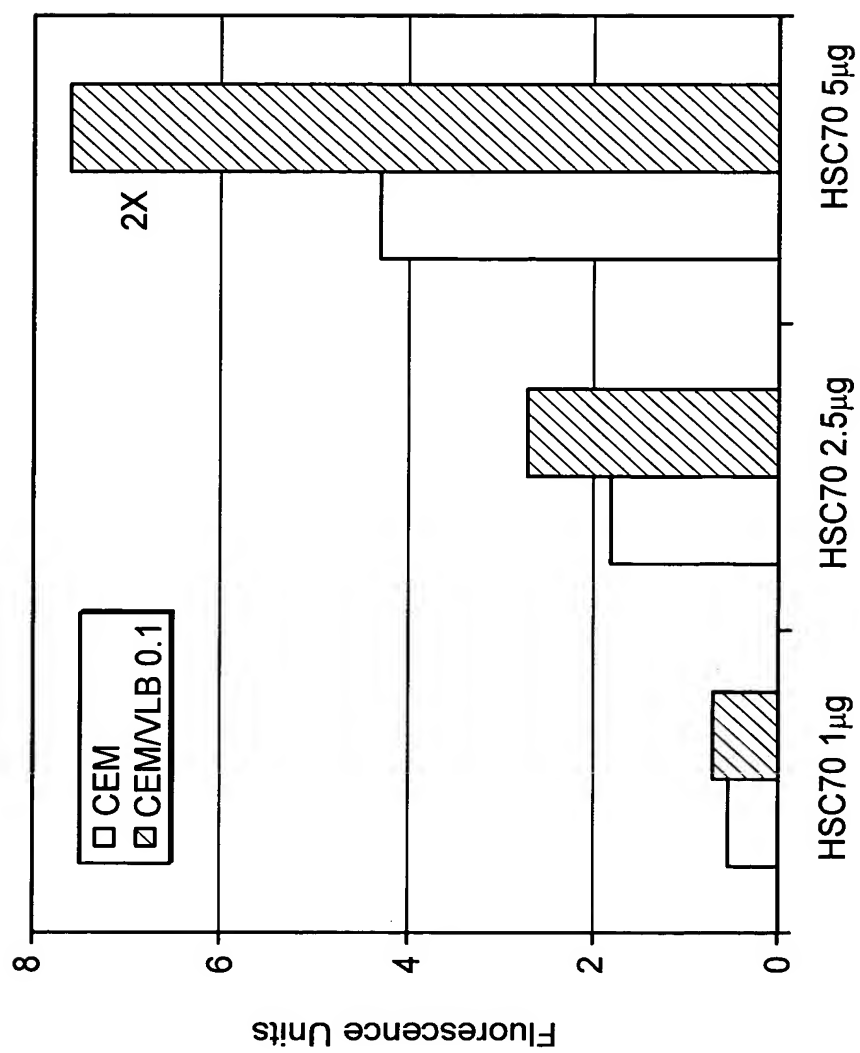


FIG. 11A

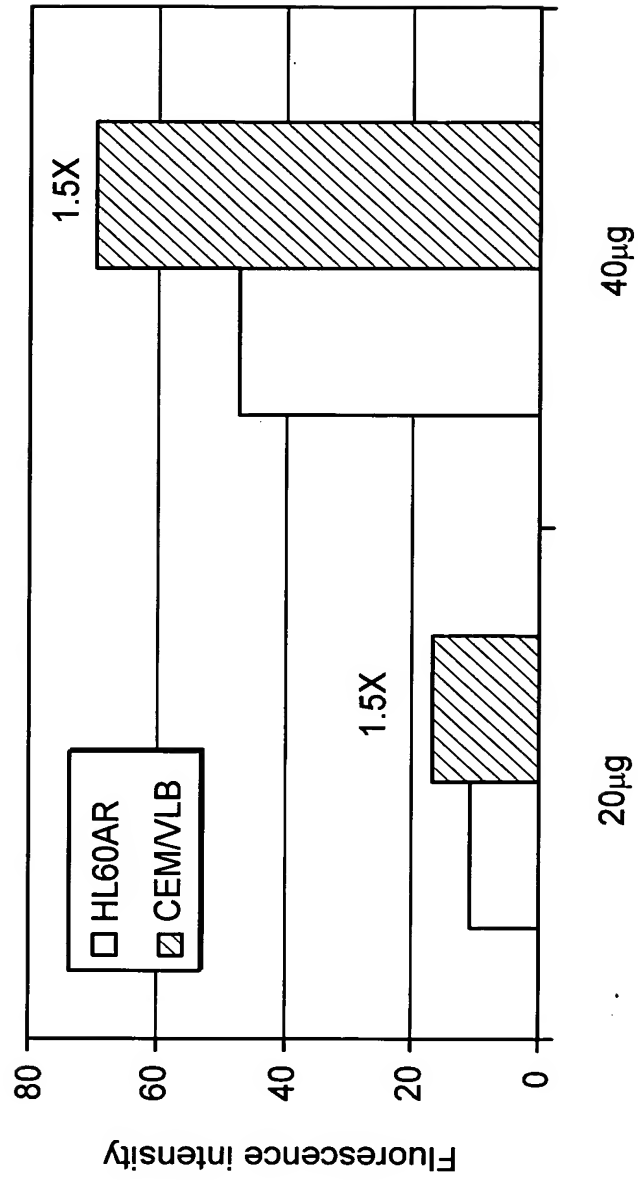


FIG. 11B

17/28

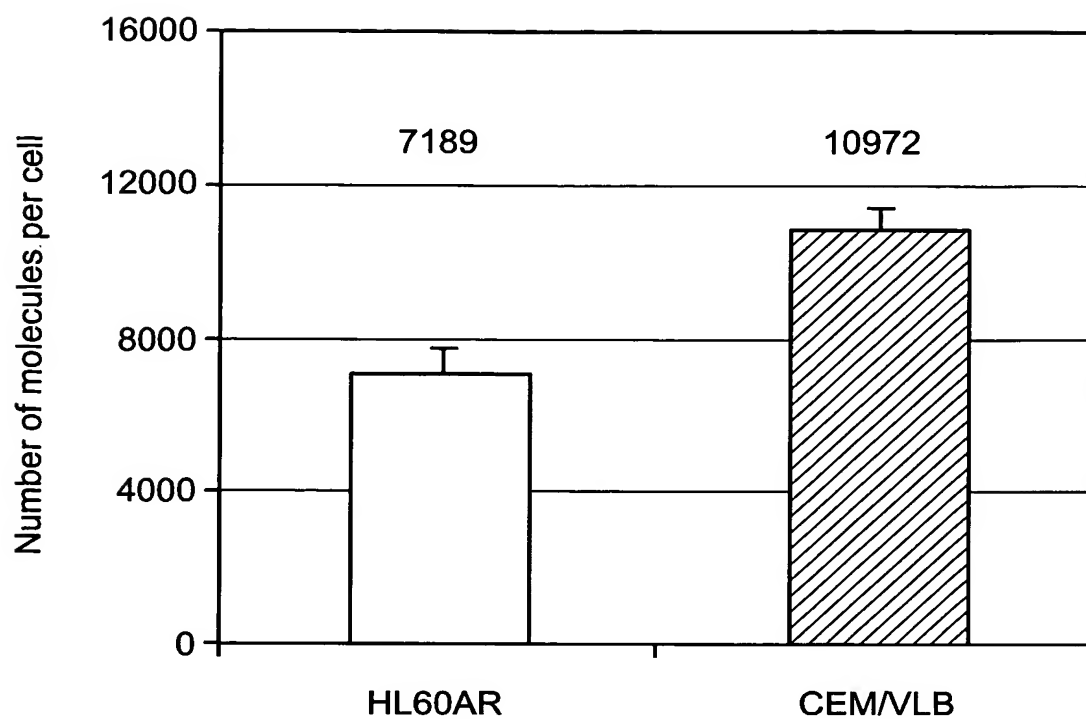


FIG. 11C

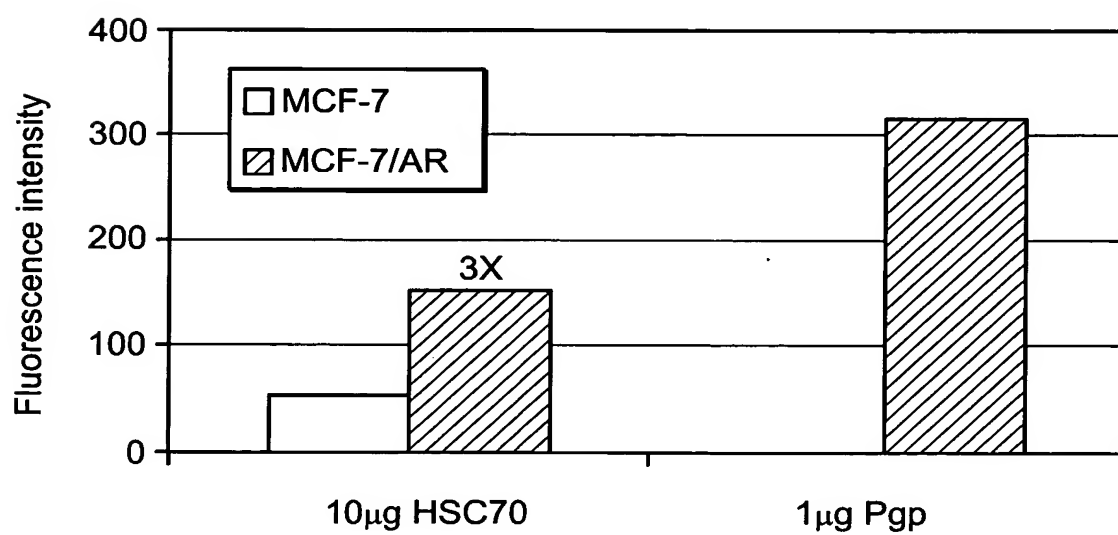


FIG. 12A

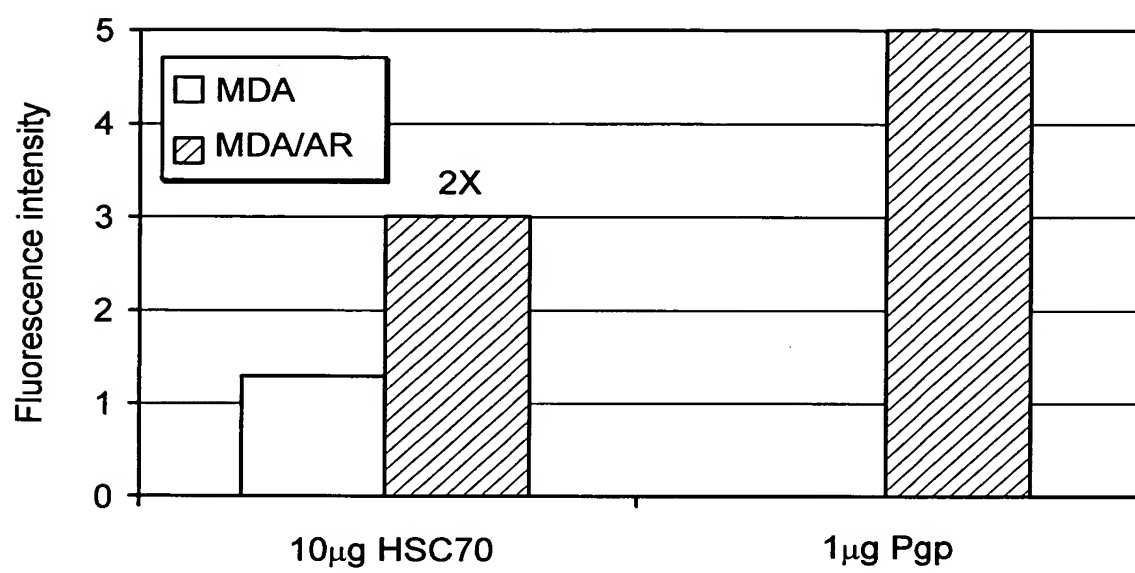


FIG. 12B

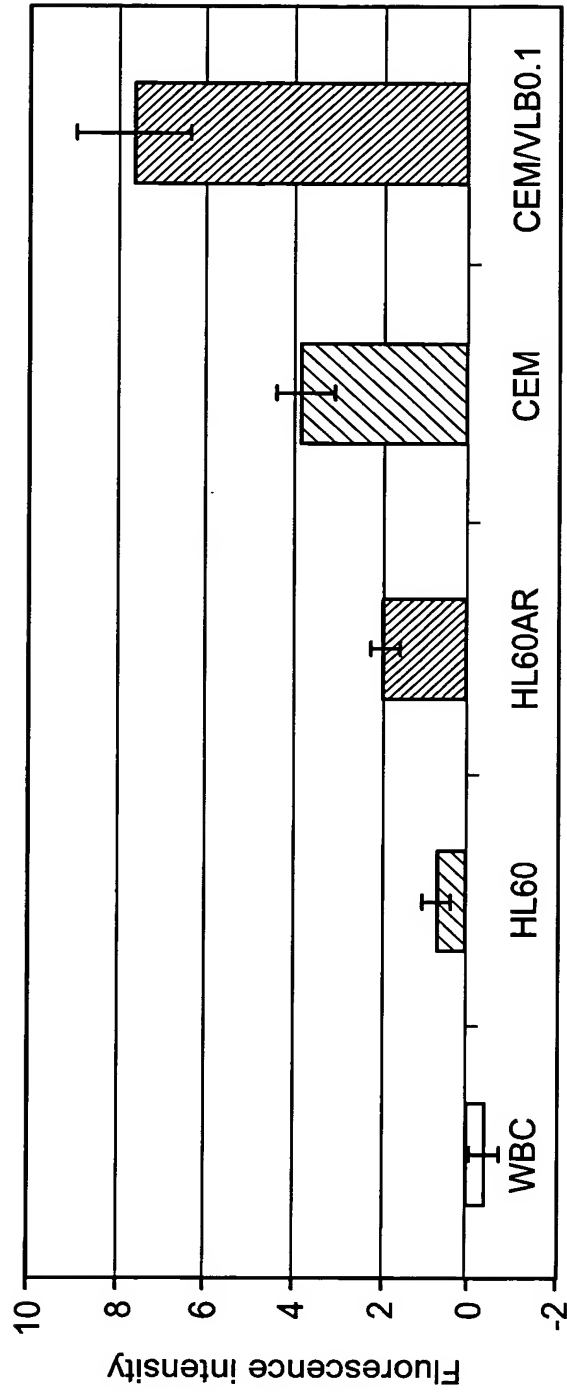


FIG. 13A

**POLYPEPTIDE SEQUENCE OF HUMAN HSC70
(GENBANK ACCESSION NO. AAK17898 (SEQ ID NO. 1))**

1 MSKGPAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFDTERL IGDAAKNQVA
61 MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR PKVQVEYKGE TKSFYPEEVS
121 SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF NDSQRQATKD AGTIAGLNVL RIINEPTAA
181 IAYGLDKKVG AERNVLIFDL GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH
241 FIAEFKRKHK KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
301 RFEELNADLF RGTLDPVEKA LRDAKLDSQ IHDIVLVGGS TRIPKIQKLL QDFFNGKELN
361 KSINPDEAVA YGAAVQAAIL SGDENSEVQD LLLLDVTPLS LGIETAGGVM TVLIKRNNTTI
421 PTKQTQTFTT YSDNQPGVLI QVYEGERAMT KDNLLGKFE LTGIPPAPRG VPQIEVTFDI
481 DANGILNVSA VDKSTGKENK ITITNDKGRL SKEDIERMVQ EAEKYKAEDE KQDKVSSKN
541 SLESYAFNMK ATVEDEKLQK KINDEDKQKI LDKCNEIINW LDKNQAEKE EFEHQQKELE
601 KVCNPIITKL YQSAGGMPGG MPGGFPGGGA PPSGASSGP TIEEVD

FIG. 14A

| |
|------------|
| FIG. 14B-1 |
| FIG. 14B-2 |
| FIG. 14B-3 |

FIG. 14B

**DNA SEQUENCE OF HUMAN HSC70
(GENBANK ACCESSION NO. AF352832 (SEQ ID NO. 2))**

1 ATGTCCAAGG GACCTGCAGT TGGTATTGAT CTTGGCACCA CCTACTCTTG TGTGGGTGTT
61 TTCCAGCACG GAAAAGTCGA GATAATTGCC AATGATCAGG GAAACCCGAAC CACTCCAAGC
121 TATGTCGCCT TTACGGACAC TGAACGGTTG ATCGGTGATG CCGCAAAGAA TCAAGTTGCA

FIG. 14B-1

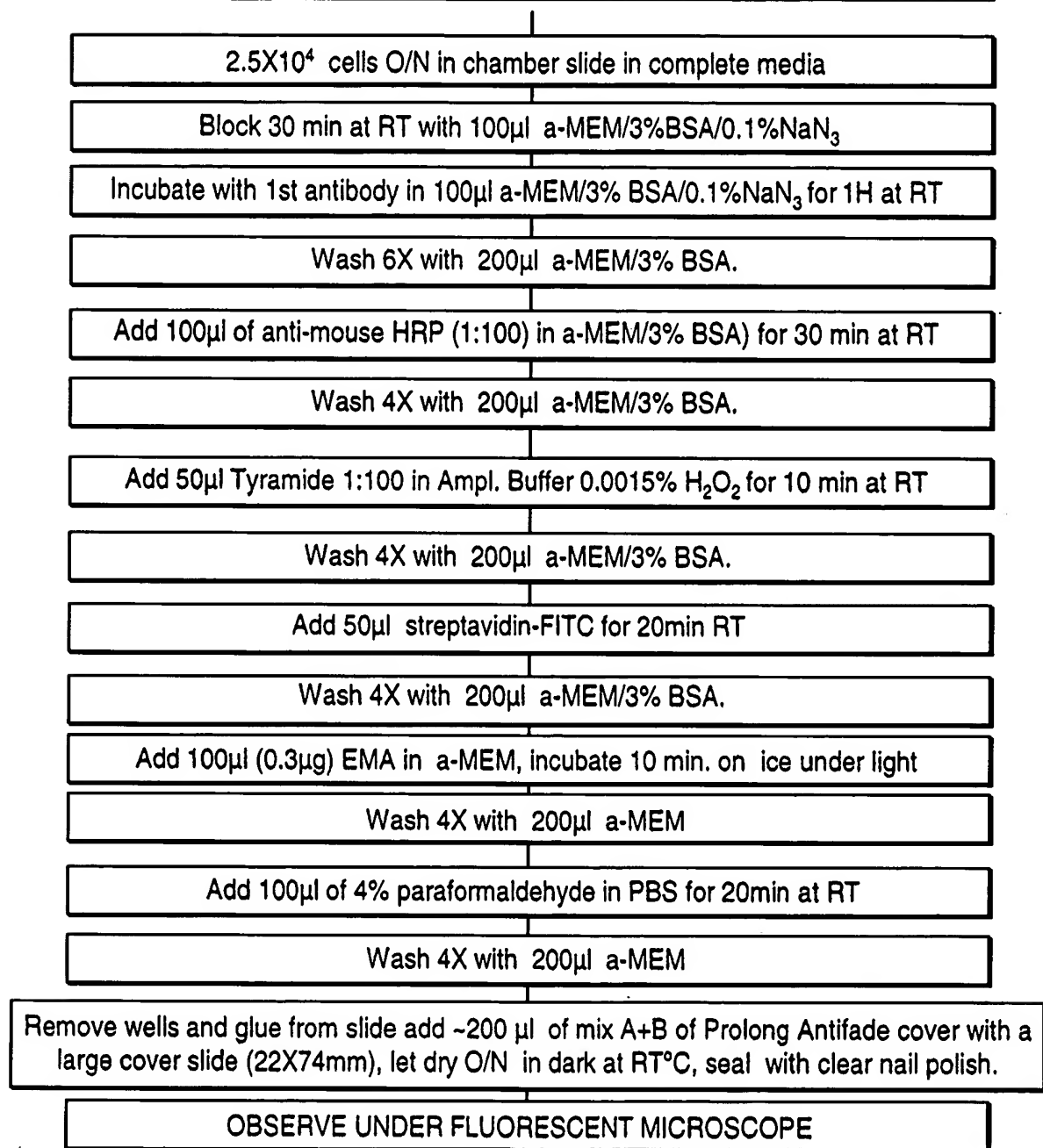
181 ATGAACCCCA CCAACACAGT TTTTGATGCC AAACGTCTGA TTGGACGCAG ATTTGATGAT
241 GCTGTTGTCC AGTCTGATAT GAAACATTGG CCCTTTATGG TGGTGAATGA TGCTGGCAGG
301 CCCAAGGTCC AAGTAGAATA CAAGGGAGAG ACCAAAAGCT TCTATCCAGA GGAGGTGTCT
361 TCTATGGTTC TGACAAAGAT GAAGGAAATT GCAGAAAGCCT ACCTTGGGAA GACTGTTACC
421 AATGCTGTGG TCACAGTGCC AGCTTACTTT AATGACTCTC AGCGTCAGGC TACCAAAGAT
481 GCTGGAACTA TTGCTGGTCT CAATGTACTT AGAATTATTA ATGAGCCAAC TGCTGCTGCT
541 ATTGCTTACG GCTTAGACAA AAAGGTTGGA GCAGAAAGAA ACGTGCTCAT CTTTGACCTG
601 GGAGGTGGCA CTTTGTGATG GTCAATCCTC ACTATTGAGG ATGGAATCTT TGAGGTCAAG
661 TCTACAGCTG GAGACACCCA CTGGGGTGA GAAGATTTTG ACAACCGAAT GGTC AACCAT
721 TTTATTGCTG AGTTTAAGCG CAAGCATAAG AAGACATCA GTGAGAACAA GAGAGCTGTA
781 AGACGCCCTC GTA CTGCTGTTG TGAACGTGCT AAGCGTACCC TCTCTTCCAG CACCAGGCC
841 AGTATTGAGA TCGATTCTCT CTATGAAGGA ATCGACTTCT ATACCTCCAT TACCCGTGCC
901 CGATTGAAG AACTGAATGC TGACCTGTTC CGTGGCACCC TGGACCCAGT AGAGAAAGCC
961 CTTGAGATG CCAAACTAGA CAAGTCACAG ATTCATGATA TTGTCTTGGT TGGTGGTTCT
1021 ACTCGTATCC CCAAGATTCA GAAGCTTCTC CAAGACTTCT TCAATGGAAA AGAACTGAAT

22/28

FIG. 14B-2

1081 AAGAGCATCA ACCCTGATGA AGCTGTTGCT TATGGTGCAG CTGTCCAGGC AGCCATCTTG
1141 TCTGGAGACA AGTCTGAGAA TGTTCAAGAT TTGCTGCTCT TGGATGTCAC TCCTCTTTCC
1201 CTTGGTATTG AAAC TGCTGG TGGAGTCATG ACTGTCCTCA TCAAGCGTAA TACCACCAT
1261 CCTACCAAGC AGACACAGAC CTTCACTACC TATTCTGACA ACCAGCCTGG TGTGCTTATT
1321 CAGGTTTATG AAGGCGAGCG TGCCATGACA AAGGATAACA ACCTGCTTGG CAAGTTTGAA
1381 CTCACAGGCA TACCTCCTGC ACCCCGAGGT GTTCCTCAGA TTGAAGTCAC TTTTGACATT
1441 GATGCCAATG GTATACTCAA TGTCTCTGCT GTGACAAGA GTACGGGAAA AGAGAACAAG
1501 ATTACTATCA CTAATGACAA GGGCCGTTTG AGCAAGGAAG ACATTGAACG TATGGTCCAG
1561 GAAGCTGAGA AGTACAAAGC TGAAGATGAG AAGCAGAGGG ACAAGGTGC ATCCAAGAAT
1621 TCACTTGAGT CCTATGCCTT CAACATGAAA GCAACTGTTG AAGATGAGAA ACTTCAAGGC
1681 AAGATTAAACG ATGAGGACAA ACAGAAGATT CTGGACAAGT GTAATGAAAT TATCAACTGG
1741 CTTGATAAGA ATCAGACTGC TGAGAAGGAA GAATTTGAAC ATCAACAGAA AGAGCTGGAG
1801 AAAGTTTGCA ACCCATCAT CACCAAGCTG TACCAGAGTG CAGGAGGCAT GCCAGGAGGA
1861 ATGCCCTGGG GATTTCCTGG TGGTGGAGCT CCTCCCTCTG GTGGTGCTTC CTCAGGGCCC
1921 ACCATTGAAG AGGTTGATTA A

FIG. 14B-3

Procedure for immunofluorescence (non-permeabilized cells)

Amplification kit used:
 TSA kit #2 with
 HRP-goat anti-mouse
 IgG and Alexa fluor 488
 tyramide from molecular
 probes T-20192

FIG. 15A

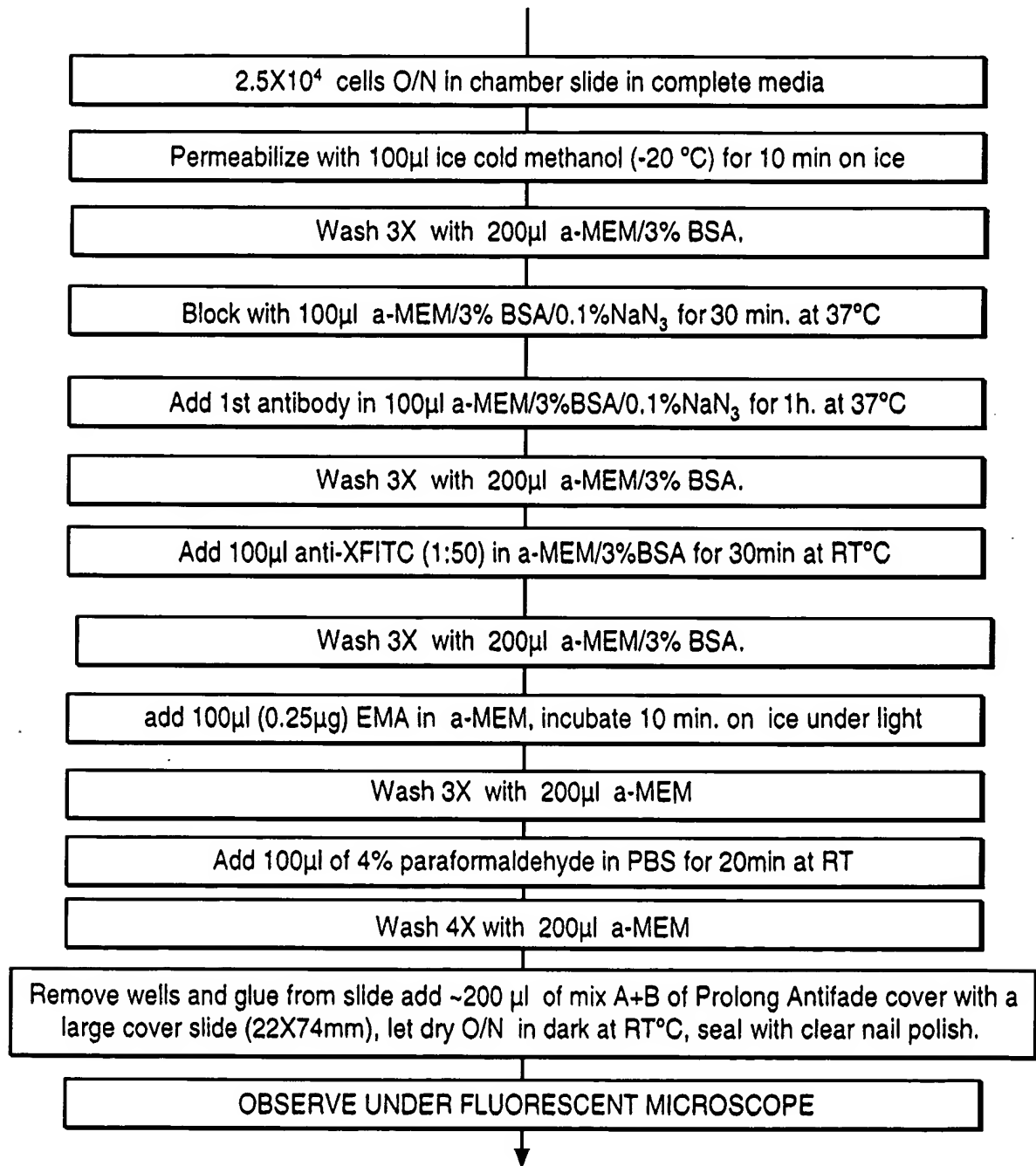
Procedure for immunofluorescence (permeabilized cells)

FIG. 15B

Immunofluorescence of surface exposed HSC70

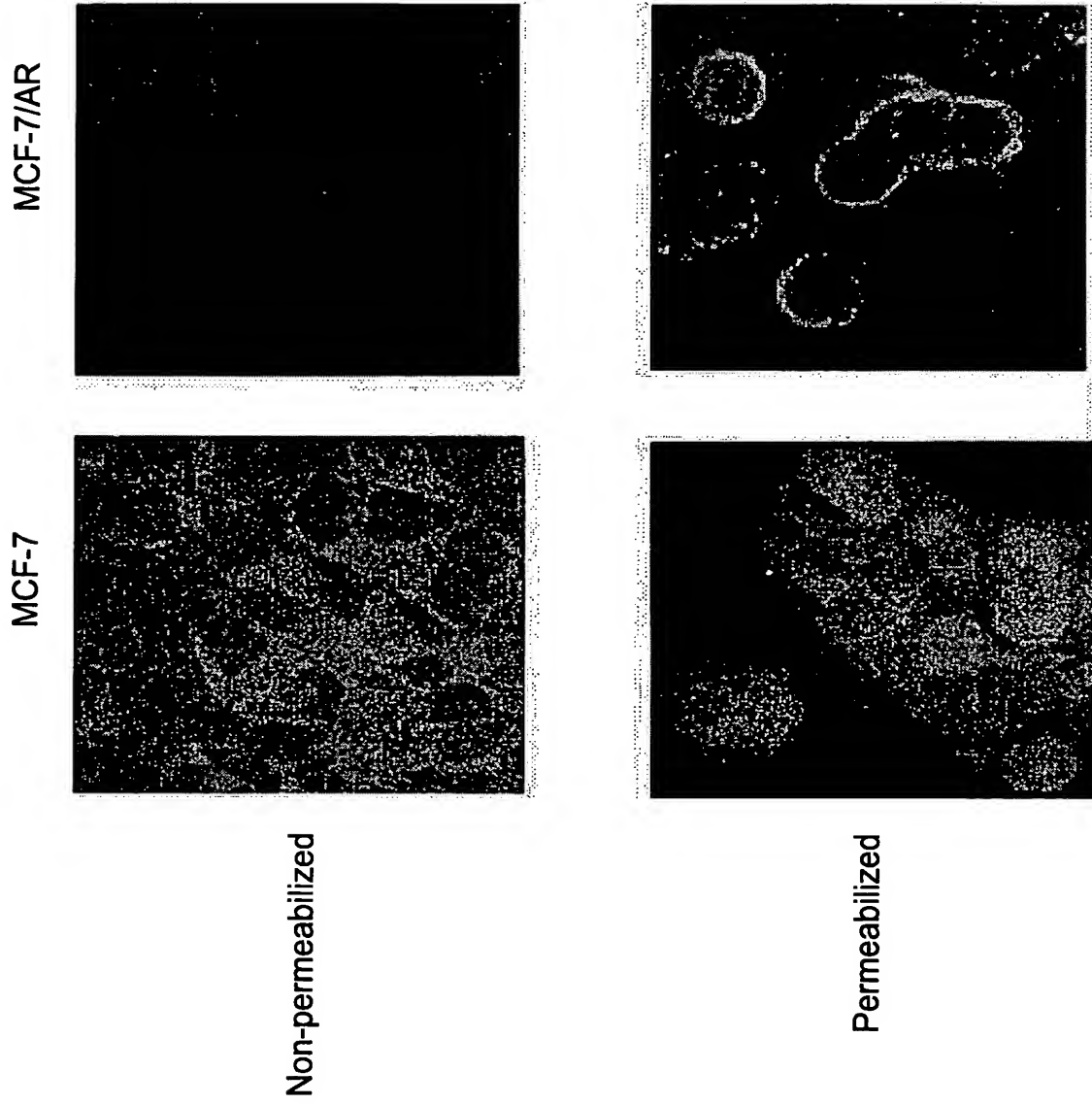


FIG. 16

HSC70 expression

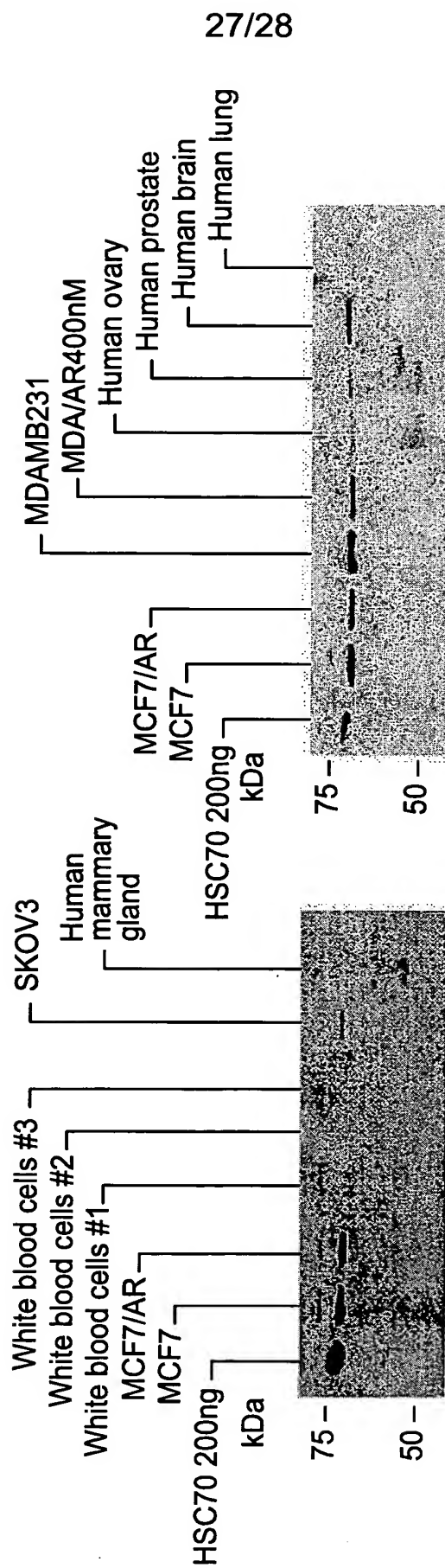


FIG. 17

FIG. 18

OVARIAN

BREAST

COLON

LUNG

Fold increase in mRNA
expression (Cy5/Cy3 ratio)

Increase of mRNA level of HSC70 in drug resistant cells compare to their related sensitive cells

